Phage selection of bicyclic peptides to challenging protein targets

Christian Heinis, Assistant Professor
EPFL, Lausanne, Switzerland

EMBO Workshop
9-12 September 2014, Hyères, France
Molecules used as therapeutics

Small molecules

+ stability
+ membrane permeability
+ oral availability
+ tissue penetration
+ chemical synthesis

> 0.5 kDa
Molecules used as therapeutics

Small molecules
+ stability
+ membrane permeability
+ oral availability
+ tissue penetration
+ chemical synthesis

Proteins
+ high affinity
+ high target specificity
+ low toxicity

> 0.5 kDa
5 - 150 kDa
Molecules used as therapeutics

Small molecules
+ stability
+ membrane permeability
+ oral availability
+ tissue penetration
+ chemical synthesis

Macrocycles
< 0.5 kDa
1 - 3 kDa

Proteins
5 - 150 kDa
+ high affinity
+ high target specificity
+ low toxicity
Bicyclic peptides
Bicyclic peptides

(Timmerman, P. et al., ChemBioChem, 2005)
Phage display technology

Phage display technology

Bicyclic peptides

(Timmerman, P. et al., ChemBioChem, 2005)
Phage selection of bicyclic peptides
Phage selection of bicyclic peptides
Phage selection of bicyclic peptides


Laboratory of Molecular Biology (LMB), Cambridge, UK

Sir Gregory Winter
Talk outline

1. **Method**: phage selection of bicyclic peptides
2. **Therapeutic application**: stability, pharmacokinetics, therapeutic targets
3. **New bicyclic peptide formats**
4. **Specific MMP-2 inhibitor**: a long-standing goal in medicinal chemistry
Talk outline

1. **Method**: phage selection of bicyclic peptides
2. **Therapeutic application**: stability, pharmacokinetics, therapeutic targets
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4. **Specific MMP-2 inhibitor**: a long-standing goal in medicinal chemistry
Urokinase-type plasminogen activator (uPA)

- Trypsin-like serine protease (secreted → extracellular)
- Overexpressed in some tumors
- Proteolytic activity was reported to promote tumor growth and metastasis formation
Phage library

library size:
> 4x10^9
transformants
Phage selections
Inhibitory activity of UK18

$K_i = 53 \text{ nM}$
Inhibitory activity of UK18

\[ K_i = 53 \text{ nM} \]
Structure of uPA-UK18 complex

In collaboration with Prof. Giuseppe Zanotti, Univ. Padua

1.9 Å resolution; > 700 Å² binding interface

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1. **Method**: phage selection of bicyclic peptides
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Towards therapeutic application of bicyclic peptides

1. Proteolytic degradation?
   Many peptides are degraded within minutes

2. Renal clearance?
   Most peptides have $t_{1/2} < 1$ hr
Towards therapeutic application of bicyclic peptides

1. Proteolytic degradation?
   Many peptides are degraded within minutes

2. Renal clearance?
   Most peptides have $t_{1/2} < 1$ hr
Proteolytic stability in plasma ex vivo

Time:

0 hr
(peptide only)

0 hr

1 hr

4 hrs

16 hrs
Towards therapeutic application of bicyclic peptides

1. Proteolytic degradation?
   Many peptides are degraded within minutes

2. Renal clearance?
   Most peptides have $t_{1/2} < 1$ hr
Elimination half-life of bicyclic peptide UK18

\[ t_{1/2} \beta = 30 \text{ min} \]
Conjugation to albumin-binding peptide

Half-life of albumin in humans = 19 days
Conjugation to albumin-binding peptide

Half-life of albumin in humans = 19 days

bicyclic peptide UK18

albumin-binding peptide
(SA21; Dennis, M., et al., JBC, 2002)
Elimination half-life

# Bicyclic peptides developed in my laboratory

<table>
<thead>
<tr>
<th>Target</th>
<th>Affinity ($K_i$ or $K_d$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cancer</strong></td>
<td></td>
</tr>
<tr>
<td>uPA (protease inhibition)</td>
<td>$K_i = 28$ nM</td>
</tr>
<tr>
<td>MMP-2 (protease inhibition)</td>
<td>$K_i = 10$ nM</td>
</tr>
<tr>
<td>CA IX (drug delivery)</td>
<td>$K_d = 24$ nM</td>
</tr>
<tr>
<td>PSA (prostate cancer diagnosis)</td>
<td>$K_d = 30$ nM</td>
</tr>
<tr>
<td>Notch1 (signalling inhibition)</td>
<td>$K_d = 170$ nM</td>
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<tr>
<td><strong>Vascular disorders</strong></td>
<td></td>
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<tr>
<td>Plasma kallikrein (edema)</td>
<td>$K_i = 0.3$ nM</td>
</tr>
<tr>
<td>Factor XIIa (thrombosis)</td>
<td>$K_i = 22$ nM</td>
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Talk outline

1. **Method**: phage selection of bicyclic peptides
2. **Therapeutic application**: stability, pharmacokinetics, therapeutic targets
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4. **Specific MMP-2 inhibitor**: a long-standing goal in medicinal chemistry
"First generation" bicyclic peptides
"First generation" bicyclic peptides

peptide format

chemical linker

<table>
<thead>
<tr>
<th>cys 1</th>
<th>cys 2</th>
<th>cys 3</th>
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</thead>
<tbody>
<tr>
<td>A C X X X X X</td>
<td>C X X X X X X</td>
<td>C G</td>
</tr>
</tbody>
</table>
Variation of the ring size
Peptides isolated against a model target (uPA)
New cyclization linkers

old

new
Peptides selected against model target (uPA)
Peptides selected against model target (uPA)
Peptides selected against model target (uPA)
Peptides selected against model target (uPA)

TBMB

TATA

TBAB
Linker swapping

<table>
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<td>0.78</td>
<td>&gt;200</td>
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<td>1.53</td>
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$K_i$ in µM
Linker swapping

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<td>&gt;200</td>
<td>0.18</td>
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<tr>
<td>&gt;200</td>
<td>0.09</td>
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$K_i$ in $\mu$M
Linker swapping

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$K_i$ in $\mu$M
Linker swapping

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<td>8.65</td>
<td>158.9</td>
<td>306.9</td>
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<tr>
<td>10.5</td>
<td>44.98</td>
<td>259.6</td>
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X-ray structure of bicyclic peptide-uPA could be determined
Bicyclic peptides bind in different orientations
Bicyclic peptides bind in different orientations
H-bonds between scaffold and peptide may stabilize binding of inhibitor
Small molecule TBAB nucleates peptide

Binding affinities

<table>
<thead>
<tr>
<th>Target Protein</th>
<th>K_d or K_i</th>
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<tr>
<td>uPA (3x3 library)</td>
<td></td>
</tr>
<tr>
<td>uPA (4x4 library)</td>
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<tr>
<td>FXIIa</td>
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<tr>
<td>actin</td>
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</table>

Chemical linkers:

- TBMB
- TATA
- TBAB
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Camille Villequey
Dr. Sangram Kale
Jonas Wilbs MMP-2

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Dr. Jeremy Touati
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Dr. Raphael Gübeli
Dr. Michal Sabisz
Dr. Vanessa Baeriswyl FXIIa

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